Effect of ouabain on CFTR gene expression in human Calu-3 cells

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Submitted 30 September 2002; accepted in final form 28 October 2002

Baudouin-Legros, Maryvonne, Franck Brouillard, Danielle Tondelier, Alexandre Hinzpeter, and Aleksander Edelman. Effect of ouabain on CFTR gene expression in human Calu-3 cells. Am J Physiol Cell Physiol 284: C620–C626, 2003; 10.1152/ajpcell.00457.2002.—We have previously shown that ouabain, which changes the electrochemical properties of cell membranes by inhibiting Na⁺,K⁺-ATPase, induces the expression of multidrug resistance (MDR-1) gene in several human cell lines. Because the expressions of the MDR-1 and CFTR (which encodes the cAMP-activated Cl⁻ channel associated with cystic fibrosis) genes are physiologically regulated in opposing directions, we wanted to determine whether ouabain also decreases CFTR transcripts and subsequently to analyze its mechanism of action. We found that the submicromolar concentrations of ouabain that increase MDR-1 mRNAs decrease the CFTR transcripts with analogous time-dependency in human pulmonary Calu-3 cells. By altering or reproducing the ouabain-induced changes in intracellular ionic activities (decreasing in external Na⁺ or K⁺ or using Na⁺ ionophore), we show that the ouabain-induced regulations of both CFTR and MDR-1 transcripts depend on the Na⁺/K⁺ pump inhibition but that the decrease in CFTR mRNAs also proceeds from cytoplasm reactions simultaneously activated by ouabain. These data, which emphasize the complex mechanism of action of ouabain, suggest that changes in intracellular ionic activities modulate CFTR/MDR-1 gene expressions.

Cystic fibrosis transmembrane conductance regulator; Na⁺,K⁺-ATPase inhibition; gene expression; transduction mechanisms

Ouabain controls the activity of most ion transporters either directly or indirectly (3) by inhibiting Na⁺,K⁺-ATPase, which supports the ionic concentration gradients between the intra- and the extracellular fluids and the electrical potential of the cell membrane (5). Recent studies have shown that it has other effects that are not clearly linked to the inhibition of transmembrane ion transport, such as altering cell growth and differentiation and triggering apoptosis (4, 14, 24). The expression of several genes is modulated in these processes, both early activated genes (c-fos, c-jun, etc.) (14, 21, 26) and late-response genes. The late-response genes encode cytosolic and secreted proteins (11) or membrane proteins (10, 15, 20, 30), including the Na⁺,K⁺-ATPase subunits, whose synthesis is controlled by ouabain in cardiomyocytes (10, 15, 30) and kidney cells (20).

Several cytosolic pathways transduce the actions of ouabain. Ouabain activates the early-response genes and the gene encoding α-skeletal actin by increasing the cytosolic Ca²⁺ concentration ([Ca²⁺]) and activating protein kinase C in rat cardiomyocytes (10, 26). However, it stimulates c-fos and Egr-1 gene transcription in human monocytic leukemia cells with no increase in [Ca²⁺] and no stimulation of protein kinase C (22). Ouabain stimulates the synthesis of the α1 and β1 Na⁺,K⁺-ATPase subunits in kidney epithelial cells via the increased intracellular Na⁺ concentration ([Na⁺]) produced by inhibiting Na⁺,K⁺-ATPase (20), and high [Na⁺] triggers the activation of MAP kinase cascades in several cell types (16). On the other hand, the stimulation of p42/44 MAP kinase in rat cardiomyocytes is independent of increased [Na⁺] (14). This increase is triggered, together with the production of reactive oxygen species (ROS), by the activation of Ras produced by the interaction of Na⁺,K⁺-ATPase and the other membrane proteins Src and EGFR (epidermal growth factor receptor) (7, 8, 18, 19, 31).

We have previously shown that ouabain stimulates the expression of the MDR-1 (multidrug resistance) gene and the synthesis of an active P-glycoprotein (P-gp) in some human epithelial cells (4), particularly pulmonary Calu-3 cells, which have the properties of the serous cells of the pulmonary submucosal glands (9). These cells are often used to study the expression of the CFTR (cystic fibrosis transmembrane conductance regulator) gene. This gene undergoes numerous mutations, causing cystic fibrosis. It encodes a transmembrane protein acting as a cAMP-activated Cl⁻ channel (27), and its expression is regulated, transcriptionally or posttranscriptionally, by several cytoplasm transduction processes, among which are the activation of protein kinase C and MAP kinase cascades (1, 35). Like P-gp, CFTR is one of the ATP binding cassette (ABC) proteins. The proteins are also linked by the fact that...

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the CFTR and MDR-1 genes are frequently regulated in opposite directions in various epithelial cells by hormones and during cell differentiation (33). The origin of the balance is still unknown. The present study was undertaken to determine whether stimulating Calu-3 cells with ouabain produced this opposing regulation of the CFTR and MDR-1 genes and whether the phenomenon was directly linked to altered ion transports.

**EXPERIMENTAL PROCEDURES**

**Cell culture and treatment.** Calu-3 cells were obtained from the ATCC and cultured on plastic in DMEM containing 1 mM sodium pyruvate, nonessential amino acids, and 10% fetal calf serum (FCS) at 37°C in a 5% CO₂-95% air atmosphere, except when otherwise noted. They were incubated with freshly prepared ouabain (Sigma-Aldrich) for the indicated time (generally 24 h). Ouabain toxicity was assessed by treating the subconfluent cultures with ouabain for 24 and 48 h and then counting the living cells (trypan blue exclusion). Some experiments were performed in buffered saline (BS) (basal composition in mM: 140 NaCl, 5 KCl, 1.1 MgSO₄, 1 CaCl₂, 0.34 Na₂HPO₄, 0.44 NaH₂PO₄, 10 Na acetate, 5 glucose, and 25 HEPES, pH 7.4), which allows ionic substitutions (140 mM NaCl was replaced by 140 mM choline chloride, while 5 mM KCl was replaced by 5 mM choline chloride). The DMEM was replaced by these warmed solutions 30 min before adding ouabain, and the cells were incubated for 24 h at 37°C in normal air.

**RNA extraction and analysis.** Confluent cells were placed in serum-free medium for 24 h and then incubated with ouabain in the absence of FCS. Total RNAs were isolated with phenol/chloroform using the Trizol reagent (Invitrogen) according to the manufacturer’s instructions, fractionated on 0.9% agarose gels (15 μg/well), and transferred to nylon membranes (Stratagene). The membranes were hybridized with 32P-labeled cDNA probes (specific activity >10⁶ cpm/μg) with the Quik Hyb solution provided by Stratagene. The CFTR probe was the 1.5-kb EcoRI-EcoRI fragment of human CFTR cDNA (a gift from Dr. Pascale Fanen, Institut de la Santé et de la Recherche Médicale U.468, Hôpital Henri Mondor, Créteil, France). The human MDR-1 probe, kindly provided by Dr. Jean-Pierre Marie (Service d’hématologie, Hôtel-Dieu, Paris, France), was a 1.5-kb EcoRI-EcoRI fragment of human MDR-1 cDNA. The β-actin cDNA probe was purchased from Oncogene Science. The mRNAs were quantified by densitometry using an ImageMaster VSD (Pharmacia-Biotech-Amersham, Orsay, France), and the amounts of CFTR mRNA were normalized to those of β-actin. All experiments were repeated at least six times.

**Statistical analysis.** When appropriate, the statistical significance of the results was checked using ANOVA or Student’s paired or unpaired t-test.

**RESULTS**

**Influence of ouabain on CFTR gene expression.** Serum-deprived confluent Calu-3 cells were treated with ouabain for 24 h, and the absence of toxicity of the treatment was checked under microscope and by trypan blue exclusion. There were 603 ± 48 thousand living cells per cm² in control cultures, and 623 ± 46 thousand living cells per cm² after incubation in 10⁻⁷ M ouabain, 601 ± 60 after incubation in 2 × 10⁻⁷ M ouabain, and 490 ± 50 after incubation with 5 × 10⁻⁷ M ouabain (n = 6). Cells incubated with ouabain (5 × 10⁻⁸ to 5 × 10⁻⁷ M) exhibited a dose-dependent decrease in CFTR transcripts (Fig. 1, left). The typical Northern blot shown at the top of the figure shows that ouabain enhanced MDR-1 gene expression in the same cells. The curves representing the changes in expression of the CFTR and MDR-1 genes quantified as CFTR and MDR-1/β-actin ratios show that about 1.5 × 10⁻⁷ M ouabain produced half inhibition of the CFTR message; this concentration also caused half-maximal stimulation of the MDR-1 gene expression.

The time course of the ouabain effect also shows the balance between expressions of the CFTR and MDR-1 genes (Fig. 1, right). The decrease in CFTR mRNA produced by 2 × 10⁻⁷ M ouabain was clear after 12 h of incubation, and the maximal inhibition was reached between 18 and 24 h. This was reflected in mirror image, by the stimulation of MDR-1 gene expression.

Preculture of the Calu-3 cells for 1 h with actinomycin D (5 μg/ml), which inhibits gene transcription and significantly reduced the amount of CFTR transcripts in the cells after 24 h, prevented the effects of ouabain on both the CFTR and MDR-1 genes (Fig. 2). However, blocking protein synthesis by cycloheximide (6 × 10⁻⁶ M) had different effects on the responses of the CFTR and MDR-1 genes (Fig. 2). It inhibited the decrease in CFTR mRNAs caused by ouabain but did not change the increase in MDR-1 mRNAs. Thus the decrease in CFTR mRNAs and the increase in MDR-1 mRNAs are due to different mechanisms, despite being triggered by the same concentrations of ouabain and having similar time courses. Ouabain appears to stimulate the MDR-1 gene directly but to control CFTR gene expression in a more complex fashion, via the synthesis of one or more intermediate protein(s).
Downregulation of CFTR Gene Expression by Ouabain

The cells were then treated with digoxin, another agent that binds to the pump specifically and also stimulates MDR-1 gene expression (4), to determine whether inhibiting Na⁺,K⁺-ATPase reduced CFTR gene activity. Digoxin also dose-dependently decreased CFTR mRNA (Fig. 3), with a time course (Fig. 3A) and an activity (Fig. 3B) very similar to those of ouabain.

We then blocked the pump by replacing extracellular K⁺ with choline to further check the link between the ouabain effect on gene expression and inhibiting Na⁺,K⁺-ATPase. The control cells were placed in physiological saline (see EXPERIMENTAL PROCEDURES) and kept at 37°C in air. Under these new experimental conditions, the ouabain effects on the expressions of the CFTR and MDR-1 genes were analogous to those measured in DMEM. Removing extracellular K⁺, which inhibits the Na⁺/K⁺ pump, neither decreased the basal cell content of CFTR transcripts nor prevented inhibition by ouabain (Fig. 4). Therefore, blocking Na⁺,K⁺-ATPase without using one of its specific inhibitors does not decrease CFTR mRNAs. Downregulation of CFTR gene expression by ouabain thus appears to involve some ion-independent action of the drug. On the other hand, as also shown in Fig. 4, cells incubated for 24 h in K⁺-free medium have three times more (312 ± 85%) of the control; n = 5) MDR-1 mRNAs than controls. This increase was analogous to that caused by 2 × 10⁻⁷ M ouabain in control medium. But cells incubated in K⁺-free medium plus ouabain showed no further increase in MDR-1 mRNAs. Thus inhibiting Na⁺,K⁺-ATPase may directly trigger ouabain-induced MDR-1 gene expression.

Ouabain thus appears to cause the opposite, acting on the expressions of the CFTR and MDR-1 genes via different reactions. Those directly linked to Na⁺,K⁺-ATPase inhibition stimulate MDR-1 gene expression.

They are not sufficient to reduce the production of CFTR mRNAs, but they may be necessary.

Involvement of ionic factors in the regulation of CFTR and MDR-1 gene expressions. Blocking the ion pump may be involved in modulating the gene expressions via alternating ion concentrations, particularly by increasing intracellular Na⁺ concentration. We checked this by replacing most of the extracellular Na⁺ by choline (leaving only 20 mM Na⁺) to prevent the increase in [Na⁺]i caused by inhibiting Na⁺,K⁺-ATPase (23). Cells incubated in low-Na⁺ medium for 24 h showed little change in CFTR gene expression, but the ouabain-induced decrease in CFTR mRNA was blocked (Fig. 5). Similarly, this ionic substitution did not significantly affect the basal cell content of MDR-1 mRNAs, but it also inhibited the ouabain-induced stimulation of MDR-1 gene expression. These data confirm that MDR-1 gene expression depends on Na⁺,K⁺-ATPase inhibition and point to increased [Na⁺]i being involved in the inhibition of CFTR gene expression by ouabain. Treating the Calu-3 cells with sodium ionophore monensin (10⁻⁶ M), added 30 min before ouabain, which corresponded to the optimal concentration, confirmed this hypothesis. In prelimi-
nary experiments, we verified that treating the cells with 10^{-6} M of monensin for the time of the experiment was not toxic (we have compared the number of living cells on one hand, and on the other, the total RNA content in the monensin-treated and untreated cultures). Monensin decreased CFTR mRNAs and stimulated MDR-1 gene expression. It also prevented any further increase in MDR-1 mRNAs by ouabain but did not prevent any additional inhibition by ouabain.

A high intracellular Ca^{2+} concentration ([Ca^{2+}]_{i}) due to increased [Na^{+}]_{i} mediates some effects of ouabain. The possibility that ouabain controls CFTR and MDR-1 gene expression via changes in [Ca^{2+}]_{i} was therefore checked using BAPTA and thapsigargin (Fig. 6). Chelating cytoplasmic calcium with BAPTA (10^{-5} M BAPTA-AM added to the culture medium), which itself decreased both CFTR and MDR-1 transcripts, prevented ouabain causing any further change in CFTR and MDR-1 gene expression. Increasing [Ca^{2+}]_{i}, with thapsigargin (2 \times 10^{-6} M, the optimal concentration) enhanced the effect of ouabain on both CFTR and MDR-1 transcripts. However, thapsigargin significantly decreased the basal amount of CFTR transcripts but not that of MDR-1 mRNAs.

Thus inhibiting Na^{+},K^{+}-ATPase and the resulting increases in cytoplasmic Na^{+} and Ca^{2+} are involved in the actions of ouabain on MDR-1 gene expression and in the decrease in CFTR mRNAs.

Cytosolic reactions involved in the regulation of CFTR gene expression by ouabain. Several cytosolic reactions triggered by ouabain are involved in modulating the expression of several genes in rat cardiomyocytes. Among these are the activation of protein kinase C and p42/44 MAP kinase cascade (8, 14, 19) and the production of ROS (18, 34). The present research was not designed to analyze these ouabain-induced reactions, and we simply checked their involvement in the control of CFTR and MDR-1 gene expression by Northern blotting.

The modulation of both genes by ouabain expressions was first explored using inhibitors of the protein kinase C and p38 and p42/44 MAP kinase cascades. We used concentrations that did not alter the viability of the Calu-3 cells and found that only PD 98059, the p42/44 MAP kinase cascade blocker, inhibited the decrease of CFTR transcripts caused by ouabain (Table 1). The stimulation of MDR-1 gene expression by ouabain remained unchanged in all the experiments. We checked the role of ROS that might have been formed in response to ouabain stimulation by treating the Calu-3 cells with N-acetylcysteine (NAC, 10^{-2} M) and diphenyleneiodonium (DPI, 2 \times 10^{-5} M), which inhibit this ROS production at various levels. Both products inhibited the decrease in CFTR mRNAs...
Table 1. Effect of kinase inhibitors on the regulation of CFTR and MDR-1 gene expressions by ouabain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CFTR mRNAs</th>
<th>MDR-1 mRNAs</th>
</tr>
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<tbody>
<tr>
<td>DMEM alone</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+ ouabain (2 × 10⁻⁷ M)</td>
<td>48 ± 8*</td>
<td>276 ± 37*</td>
</tr>
<tr>
<td>BIM (10⁻⁷ M)</td>
<td>104 ± 7</td>
<td>105 ± 9</td>
</tr>
<tr>
<td>+ ouabain</td>
<td>50 ± 9 NS</td>
<td>285 ± 23 NS</td>
</tr>
<tr>
<td>SB-203580 (2 × 10⁻⁵ M)</td>
<td>78 ± 14</td>
<td>81 ± 14</td>
</tr>
<tr>
<td>+ ouabain</td>
<td>43 ± 12 NS</td>
<td>267 ± 34 NS</td>
</tr>
<tr>
<td>PD-98059 (2 × 10⁻⁵ M)</td>
<td>95 ± 13</td>
<td>79 ± 11</td>
</tr>
<tr>
<td>+ ouabain</td>
<td>72 ± 12 NS</td>
<td>221 ± 15 NS</td>
</tr>
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</table>

The Calu-3 cells were treated with bisindoylmaleimide (BIM), SB-203580, or PD-98059, which inhibit protein kinase C, p38 MAP kinase, and the p42/44 MAP kinase cascade, respectively, 15 min before adding 2 × 10⁻⁷ M ouabain. The CFTR and multidrug resistance (MDR)-1 transcripts in the cells 24 h later were estimated from the CFTR (or MDR-1)/β-actin ratios expressed as percentages of the values found in controls. The values are the means ± SE of 4 determinations. Two statistical analysis comparisons were performed. The effect of ouabain was first assessed by comparing the means of the results for ouabain-treated cells and their controls using Student’s unpaired t-test; these results are represented by the first indicator after each entry (NS, P > 0.5; *P < 0.05). The modifications of the ouabain effect brought about by the kinase inhibitors were then assessed by comparing the control and ouabain-treated cells in the absence and presence of each inhibitor using Student’s paired t-test; these results, if applicable, are represented by the second indicator after each entry (NS, P > 0.5; †P < 0.05).

caused by ouabain but not the stimulation of MDR-1 gene expression (Fig. 7).

The above data on the effect of inhibiting Na⁺,K⁺-ATPase on the way ouabain regulates the expression of the CFTR and MDR-1 genes thus show that inhibiting the pump results in increases in MDR-1 transcripts more directly than decreases in CFTR mRNAs. Blocking the regulation of CFTR gene expression by ouabain with inhibitors of cytoplasmic transduction pathways confirmed this notion.

**DISCUSSION**

Submicromolar concentrations of the specific Na⁺,K⁺-ATPase inhibitor, ouabain, which stimulate MDR-1 gene expression and P-gp synthesis, also decrease the concentrations of CFTR transcripts in the human Calu-3 cells. The pulmonary cells used in this study have the characteristics of the serous cells of the submucosal pulmonary glands devoted to mucous secretion (9).

We used ouabain concentrations in the same range as those that act on other human cells to decrease the synthesis of membrane proteins in T-84 colon cells (28), stimulate ROS production in HeLa cells (18), and modulate the expression of several genes in THP-1 (human monocytic) cells (22). The time course for the loss of CFTR mRNAs is also similar to that of the late changes caused by ouabain in the expression of genes encoding membrane or cytosolic proteins in cardiomyocytes or PC-12 cells (10, 30). Therefore, the CFTR gene, like the MDR-1 gene, is one of the genes whose expression is affected by ouabain.

Ouabain decreases the amount of CFTR mRNAs while stimulating MDR-1 gene expression and P-glycoprotein (P-gp) synthesis in Calu-3 cells. This replacement of CFTR by P-gp has been described in response to both pharmacological stimulation and under physiological conditions, particularly during cell differentiation (33). The expressions of many other genes are affected during cell differentiation, and changes in Na⁺,K⁺-ATPase α-subunit isoforms and ouabain affinity are often observed (25). As there are several endogenous Na⁺,K⁺-ATPase inhibitors whose physiology remains unclear (29), our results raise the possibility of a link between the modulation of the CFTR, MDR-1, and Na⁺,K⁺-ATPase gene expressions. For example, the α1⁺-subunit is replaced by the α2⁺-subunit with a higher affinity for ouabain in the pulmonary tract at birth (13), together with decreased CFTR activity (32).

The control of the expressions of CFTR and MDR-1 genes expression by ouabain raises two problems. The first concerns the control of gene expression by the altered electrochemical properties and transmembrane ion gradients caused by inhibiting Na⁺,K⁺-ATPase. The second problem is that of the existence and the nature of a common intracellular signal that affects the expressions of the CFTR and MDR-1 genes in opposite fashions.

Inhibition of Na⁺,K⁺-ATPase alone appears to increase the cell content of MDR-1 transcripts, because incubating the cells in K⁺-free medium or with ouabain or digoxin all had the same effect of increasing MDR-1 mRNAs. Our studies on the inhibition of RNA or protein synthesis indicate that the ouabain-induced increase in MDR-1 transcripts results from the direct stimulation of gene transcription. Similar direct stimulation of early-activated gene transcription by ouabain also occurs in rat cardiomyocytes (23, 26) and in human fibroblasts, in which time course is the same as that for MDR-1 gene stimulation in Calu-3 cells (21). Like MDR-1, these early-activated genes are not expressed in quiescent cells, and proteins they encode are directly involved in the cell response to extracellular

![Fig. 7. Role of reactive oxygen species (ROS). Serum-deprived cells were incubated for 24 h under control conditions or in the presence of N-acetylcysteine (NAC; 10⁻² M) or diphenyleneiodinium (DPI; 2 × 10⁻⁴ M), with or without 2 × 10⁻⁷ M ouabain, added 30 min after the other drugs. The Northern blots were quantified and the CFTR/β-actin (A) and MDR-1/β-actin (B) mRNA ratios are expressed as percentages of the ratios found under control physiological conditions. The values are the means ± SE of 4 determinations. Effect of ouabain: *P < 0.05.](http://ajpcell.physiology.org/doi/10.1152/ajpcell.00353.2003)
stresses. This characteristic may be linked to the direct stimulation of their expression when Na\textsuperscript{+},K\textsuperscript{+}-ATPase is inhibited. In contrast, the CFTR gene codes for a constitutive protein, and it may be regulated via several mechanisms, coordinated to maintain correct cell metabolism and function. The lack of effect of the K\textsuperscript{+}-free medium indicates that simply inhibiting Na\textsuperscript{+},K\textsuperscript{+}-ATPase cannot cause the inhibition of the CFTR gene, as this process is complex and involves intermediate protein(s). Nevertheless, if not sufficient, Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibition appears to be necessary for inhibiting CFTR gene expression. This was suggested by the effect of digoxin, another Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibitor, which reproduced the ouabain effect. Moreover, neither the decrease of the CFTR transcripts by ouabain nor the stimulation of MDR-1 gene expression occurred when cells were placed in low-Na\textsuperscript{+} medium, because blockade of the pump by ouabain cannot increase intracellular the Na\textsuperscript{+} concentration. An increase in [Na\textsuperscript{+}], caused by inhibiting Na\textsuperscript{+},K\textsuperscript{+}-ATPase or by monensin, can directly stimulate the transcription of early-activated gene (22). It may also, either directly or indirectly, control the expression of late response genes, such as those encoding angiotensin II receptor 2 (30), IL-1\textbeta (22), and Na\textsuperscript{+},K\textsuperscript{+}-ATPase subunits (20). In the Calu-3 cells, this increased [Na\textsuperscript{+}], thus appears to be the common trigger of the opposing responses of the MDR-1 and CFTR gene expressions to ouabain, which could also be seen with monensin. However, the results obtained with monensin also show that increased [Na\textsuperscript{+}], alone cannot support the decrease in CFTR transcripts caused by ouabain, which remains active in the presence of the ionophore. Little is known about how high [Na\textsuperscript{+}], may directly modulate gene expression, but the effects of this parameter have been linked to a secondary increase in [Ca\textsuperscript{2+}]. Our results obtained with BAPTA and thapsigargin are consistent with such a relationship in the Calu-3 cells, because BAPTA prevents the effects of ouabain on the expression of the CFTR and MDR-1 genes and thapsigargin enhances it. Thapsigargin alone does not modify the “basal” amount of MDR-1 mRNAs, although it decreases that of CFTR transcripts. This points to a complex regulation of CFTR gene expression involving some calcium-sensitive cellular pathway(s), which could also be activated by ouabain independently of Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibition (8).

Indeed, the absence of any change in CFTR mRNAs when extracellular K\textsuperscript{+} is removed and the conservation of the ouabain effect under these conditions clearly show that other reaction(s), besides the inhibition of Na\textsuperscript{+},K\textsuperscript{+}-ATPase, are required to inhibit CFTR gene expression. Modulating gene expression by ouabain involves both increased [Ca\textsuperscript{2+}], and the ion-independent activation of protein kinase C, of p42/44 MAP kinase cascade, and of ROS production in rat cardiomyocytes (31). Pharmacological inhibition of protein kinase C and the p38 MAP kinase cascade did not alter the effects of ouabain on either gene, but blocking the p42/44 MAP kinase cascade decreased the inhibition of CFTR gene expression by ouabain without affecting MDR-1 gene transcription. This MAP kinase cascade is activated by ouabain, in a Na\textsuperscript{+}-dependent fashion, in several cells (17). Its activation by ouabain in Calu-3 cells was very slight and short-lived (<30 min, result not shown) and thus difficult to link to the decrease in CFTR transcripts.

However, inhibiting ROS production, which did not affect MDR-1 mRNAs, blocked the ouabain-induced regulation of CFTR gene expression. ROS formation, which occurs in response to ouabain independently of Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibition (18), may thus be the second stimulus for decreasing CFTR transcripts. ROS are now considered to be a second messenger in several pathways involved in the control of gene expression (6). They may also be implicated in the induction of IL-1 gene expression (12), which is also stimulated by ouabain (22). Little is known about their precise molecular actions, but they are usually part of the overall cell response to extracellular stresses. This supports their implication in the inhibition of CFTR gene expression, which commonly occurs in response to extracellular stimulation (1, 2).

We conclude that ouabain and other Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibitors regulate the expression of CFTR and MDR-1 genes in opposing senses. Inhibiting Na\textsuperscript{+},K\textsuperscript{+}-ATPase can induce MDR-1 gene expression by itself and is also necessary to decrease CFTR mRNAs. However, the downregulation of CFTR gene expression by ouabain is a complex phenomenon that also involves transduction pathways triggered by the interaction of ouabain with the Na\textsuperscript{+}/K\textsuperscript{+} pump. This illustrates both the control of gene expression by Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity and [Na\textsuperscript{+}], and the wide spectrum of action of the ouabain-like drugs.

This work was supported by Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Association Vaincre la Mucoviscidose, and Association ABCF protéines. F. Brouillard and A. Hinzpeter were supported by fellowships from the Association Vaincre la Mucoviscidose.

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DOWNREGULATION OF CFTR GENE EXPRESSION BY OUABAIN

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